DETECTION OF LOW LEVELS OF MICROBIAL CONTAMINATION ON SURFACES BY CHEMICAL APPROACHES

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INTRODUCTION

Studies are in progress to develop chemical methods which will quickly enumerate surface microbial contamination. At the present stage of development a microscope photometer is being used in order to measure and evaluate the quantities of the separated nucleotides from the microbial cells found on thin-layer plates.

OBJECTIVE

The objective of these studies is to make it possible to use the developed chemical method for the determination of the number of microbial cells on surfaces.

EXPERIMENTAL PROCEDURE

As was previously reported, nucleotides were extracted from microbial cells and separated by thin-layer chromatography. The developed chromatoplates were examined visually under ultraviolet light at 3600 and 2500 angstroms. In order to increase the sensitivity of the chemical methods, that is,to detect a smaller number of microbial cells on surfaces, a microscope photometer is being used. This unit measures the intensity of the fluorescent light which is reflected from the separated nucleotides--adenosine triphosphate.

At the present time, the chemical procedure and the microscope photometer are under a strict standardization process. This process includes:

1. Analyzing the prepared and chromatographically purified blank chromatoplates for interfering substances and the homogeneity of the layer.

2. Conducting studies which check the background chemical contamination from the surrounding environment.
3. Taking readings from the separated fluorescent nucleotides on thin-layer plates. These plates are prepared from bacteria E. coli in growth phase, Bacillus subtilis var. niger and standard compounds.

Some changes have been introduced in order to transfer the E. coli cells onto the chromatographic plate:

1. The bacteria E. coli cells were removed from the surface of the agar plate with a tarred glass capillary loop and were then weighed on an analytical balance. The weighed material was immediately transferred to distilled water, mixed thoroughly and spotted on thin-layer plates for chemical separation.
2. The cell-cleaning procedure (washing with distilled water) was omitted for the time being until certain questions can be clarified.

Besides using E. coli cells which were cultivated on the three different kinds of media mentioned above, limited work was also done with Bacillus subtilis var. niger.

The quantity of E. coli used in this investigative phase was large enough to make it possible to visually locate the significant substances on thin-layer plates in ultraviolet light. In this case the concentration was: one lambda equal to 1×10^{-6} grams. The concentration of the next group of dilutions prepared was: one lambda equal to 1×10^{-9} grams. These dilutions were applied to chromatographic plates in quantities of three, two and one lambda.

RESULTS AND DISCUSSION

The results which were obtained by using the microscope photometer are very encouraging. At present, we are not supplying groups of numbers and charts because the purpose of this phase was to standardize the method and the unit itself. Results were observed only on a readout unit. In the near future they will be recorded with a connected recorder.

The baseline for the blank, prepared chromatoplate, according to the microscope photometer readout unit, is within ±2%. This deviation from a straight line is caused by the reflection of the particles on the thin-layer and will be lowered by adding some additional material in order to

make the surface more even. This baseline is quite good and can be tolerated if it is not contaminated by the environment.

The chemical background contamination was definitely visible after the above scanned plates were exposed to a natural laboratory environment for the length of time which is required in regular procedure. On the readout unit the peaks produced from the contaminated particles were as high as 20% and covered longer or shorter distances according to the size of the particles which were deposited from the air. This proves that after the plates are chromatographically cleaned they must be protected from chemical contamination. For the same reason certain phases of the separation procedure must be conducted in a controlled environment.

The readout unit registered definitely every area where the nucleotides were located and the intensity of the peaks reached as much as 40%. The results were not linear because of the large quantities used. This is an indication that the unit will be able to detect small quantities of the extracted and separated nucleotides from a small number of microbial cells.

It is apparent that the E. coli cells were not washed with distilled water due to the obvious changes in chemical pattern which varied with the length of time the material was handled. The negative effect of distilled water on microbial cells is also reported in the literature.

CONCLUSION

The results indicate that the chemical method using thin-layer chromatography in connection with the microscope photometer produces very promising results in detecting low numbers of microbial cells on surfaces.

FUTURE WORK

Future work will involve completion of the research phase to determine how many bacteria there are on the surface. We will continue to standardize the microscope photometer and adjust the chemical procedure according to our needs. The quantity range of the unknowns for the given condition will be established. More work will be done with standard compounds, E. coli, Bacillus subtilis and other species.

The last phase of research will be devoted to analyzing different surfaces for the number of microbial cells present.